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ω-Hydroxylation Activity toward Leukotriene B_4 and Polyunsaturated Fatty Acids in the Human Hepatoblastoma Cell Line, HepG2, and Human Lung Adenocarcinoma Cell Line, A549

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The addition of glucose to the culture medium of HepG2 or A549 cells for 22 h caused a dose-dependent increase in leukotriene B_4 ω-hydroxylation activity in the homogenate. The addition of genistein to the culture medium of HepG2 or A549 cells for 22 h caused a dose-dependent decrease in the activity, although the number of living cells was not influenced by the addition of genistein. The inhibition by genistein was reversed by removal of genistein from the culture medium in 22 h. The specific leukotriene B_4 ω-hydroxylation activity was high in the nuclear envelope fraction of HepG2 or A549 cells, and a large portion of the activity was concentrated in the nuclear envelope fraction. In the nuclear envelope fraction, leukotriene B_4 ω-hydroxylation activity was accompanied by high polyunsaturated fatty acid ω-hydroxylation activity. The apparent K_m values for arachidonic acid and leukotriene B_4 in the fractions of HepG2 or A549 cells were 25 and 50 μM, or 22 and 66 μM, respectively. The V_{max} values were 222 and 104 pmol/min/mg protein, or 175 and 370 pmol/min/mg protein, respectively. NADPH-dependent ω-hydroxylation of LTB_4 in the nuclear envelope fraction of HepG2 or A549 cells was strongly inhibited by metyrapone and CO. The expression of cytochrome P450 4F2 mRNAs was detected in HepG2 and A549 cells, and thus the arachidonic acid and leukotriene B_4 ω-hydroxylation activities in the nuclear envelope fractions of HepG2 and A549 cells are likely due to cytochrome P450 4F2.

Key words: A549 cell, cytochrome P450 4F2, fatty acid ω-hydroxylation, HepG2 cell, nuclear envelope.

ω-Hydroxyeicosatetraenoic acid in the various arachidonic acid [20:4(*n*-6)] metabolites generated by the cytochrome P450 (CYP) has perhaps attracted the most interest (*1*). This ω-hydroxylated 20:4(*n*-6) derivative exhibits potent biological effects on renal tubular and vascular functions, and on the long-term control of arterial pressure (*2*).

In the human kidney or liver, CYP4A11 has been identified as the ω-hydroxylation enzyme for fatty acids (*3*, *4*). For CYP4A11, low or no ω-hydroxylation activity toward 20:4(*n*-6) has been demonstrated, compared with lauric acid (12:0) (*3–5*). On the other hand, a CYP4F2 cDNA has been isolated from human liver that exhibits extensive sequence homology (87%) with a human neutrophil CYP4F3 cDNA (*6*). Microsomes from yeast transfected with this CYP4F2 cDNA exhibit substantial leukotriene B_4 (LTB_4) 20-hydroxylase activity, but no activity has been detected toward 12:0, 20:4(*n*-6), or prostaglandin A_1 (*6*, *7*). In contrast, the purified CYP4F2 from human liver microsomes was found to catalyze the ω-hydroxylation of not only LTB_4 but also 20:4(*n*-6) (*8*, *9*). Whether or not CYP4F2 exhibits substantial ω-hydroxylase activity toward polyunsaturated fatty acids such as 20:4(*n*-6) in a human single cell line is a point of interest.

The authors found that a homogenate of a human colonic well-differentiated adenocarcinoma or a human colonic adenocarcinoma cell line, Caco-2, exhibits highly active NADPH-dependent ω-hydroxylation of docosahexaenoic acid [22:6(*n*-3)] or 20:4(*n*-6), compared with 12:0 (*10*, *11*). In addition, a homogenate of a human colonic well-differentiated adenocarcinoma exhibited high NADPH-dependent ω-hydroxylation of LTB_4 (*12*). Although purification of the ω-hydroxylation enzyme in the homogenate was not achieved, the enzyme appears to differ from CYP4A11 (*10*, *11*, *13*). For determination of the subcellular localization and alteration of polyunsaturated fatty acid/LTB_4 ω-hydroxylation activity in a human single cell line, cell lines in which high ω-hydroxylation of polyunsaturated fatty acids occurs were selected preliminarily from among several human carcinoma cell lines, and a human hepatoblastoma cell line (HepG2) and a human lung adenocarcinoma cell line (A549) were selected for this study. In the present article, we describe that LTB_4 and polyunsaturated fatty acid ω-hydroxylation activities due to CYP4F2 exists in the nuclear envelope fraction, and that the activity is affected by the extracellular genistein and glucose levels.

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Abbreviations: CYP, cytochrome P450 [EC 1.14.14.1]; 20:4(*n*-6), arachidonic acid; 12:0, lauric acid; 22:6(*n*-3), docosahexaenoic acid; LTB_4, leukotriene B_4; 16:0, palmitic acid; FCS, fetal calf serum; SGF, serum growth factor; TKM, 25 mM KCl–5 mM $MgCl_2$–50 mM Tris-HCl; RT-PCR, reverse transcriptase–polymerase chain reaction; G6P, glucose-6-phosphate.

MATERIALS AND METHODS

Materials—Prostaglandin F_2 α-3,3,4,4-$^2H_4$ and 15(*S*)-hydroxyeicosatetraenoic acid-5,6,8,9,11,12,14,15-$^2H_8$, as internal standards, and LTB_4 were obtained from Cayman Chemicals (Ann Arbor, MI, USA). Kanamycin sulfate, glucose, genistein, sodium orthovanadate, human insulin, *R*(–)epinephrine and β-estradiol were from Wako Pure Chemical Industries (Osaka). 22:6(*n*-3) and 20:4(*n*-6) were from Nu-Chek Prep. (Elysian, MN, USA). Palmitic acid (16:0) and 12:0 were from P-L Biochemicals (Milwaukee, WI, USA). ω-Hydroxyhexadecanoic acid was from Aldrich Chemical (Milwaukee, WI, USA). NADPH was from Oriental Yeast (Osaka). Metyrapone, porcine pancreas glucagon and Hoechst 33258 for the DNA assays were from Sigma (St. Louis, MO, USA). The BCA Protein Assay Kit was from Pierce (Rockford, IL, USA). CO gas (purity, 99.9%) was from Nippon Sanso (Tokyo). All other chemicals were from Wako Pure Chemical Industries.

HepG2 and A549 Cell Lines—HepG2 and A549 cells (ATCC, Rockville, MD, USA), respectively, were grown in 25 cm^2 flasks containing Eagle's MEM medium (Nihon Pharmaceutical, Osaka) including 1,000 mg/liter glucose, 50 mg/liter kanamycin sulfate and 10% fetal calf serum (FCS; Biological Industries, Kibbutz Beit Haemek, Israel), or Git medium [Daigo's T medium containing 3151 mg/liter glucose and 10% cow serum growth factor (Daigo GF-21); Nihon Pharmaceutical] at 37°C in a 5% CO_2 incubator. The medium was changed every 3 days.

To examine the effect of medium exchange, Eagle's MEM medium at 7 days subculture of HepG2 cells or 13 days subculture of A549 cells, was replaced with Git medium for 22 h. To examine the effect of the glucose concentration in the culture medium, the medium, at 7 days subculture of HepG2 cells or 13 days subculture of A549 cells, was replaced with Eagle's MEM medium containing 1,000, 1,308, 1,613, or 2,215 mg/liter glucose for 22 h. To examine the effect of genistein (tyrosine-kinase inhibitor) or orthovanadate (tyrosine-phosphatase inhibitor) in the culture medium, Git medium at 7 days subculture of HepG2 cells or 13 days subculture of A549 cells, was replaced with Git medium containing 0, 15, 30, and 60 μM genistein or 0, 50, 100, and 200 μM sodium orthovanadate for 22 h. The number of living cells was determined by the 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt–substrate method using a Cell Counting Kit-8 as specified by Wako Pure Chemical Industries. To examine reversal the genistein effect, the medium (which had already been replaced with Git medium containing 60 μM genistein for 22 h at 6 days subculture of HepG2 cells) was replaced with only Git medium for 22 h. To examine the effect of insulin, glucagon, *R*(–)epinephrine, or β-estradiol in the culture medium, the medium (Git medium) at 7 days subculture of HepG2 cells or 13 days subculture of A549 cells was replaced with that containing 5 μM insulin, 100 nM glucagon, 10 μM *R*(–)epinephrine, or 61 μM β-estradiol for 22 h. The cells were mechanically scraped off in Hank's balanced salt solution, washed with the same solution and then homogenized with a Polytron-homogenizer in 1.5 ml of ice-cold 50 mM Tris-HCl buffer (pH 8.0) for 30 s.

To determine the subcellular localization of ω-hydroxylation activity, HepG2 or A549 cells were grown in 75 cm^2 flasks containing Git medium for 7 days after subculture of HepG2 cells or for 14 days after subculture of A549 cells. HepG2 cells were removed from two 75 cm^2 flasks, washed as above and then homogenized (up and down 5 times at 700 rpm) with a Potter-Elvehjem Teflon homogenizer in 2.0 ml of 250 mM sucrose–50 mM KCl–2 mM $MgCl_2$–20 mM Tris-HCl isoosmolaric buffer (pH 7.6). A549 cells were removed from two 75 cm^2 flasks, washed as above and homogenized with a Polytron-homogenizer in 2.0 ml of the isoosmolaric buffer. Each homogenate was fractionated by differential centrifugation at 100 ×*g* for 10 min, 7,000 ×*g* for 10 min and 100,000 ×*g* for 1 h at 4°C. The 100 ×*g* and 7,000 ×*g* pellets were each further fractionated by sucrose density gradient centrifugation (ρ = 1.15–1.20, 250 mM sucrose–50 mM KCl–2 mM $MgCl_2$–20 mM Tris-HCl) at 100,000 ×*g* for 2 h at 4°C. The fraction corresponding to each density was collected and subjected to centrifugation in the above isoosmolaric buffer (pH 7.6) at 100,000 ×*g* for 1 h at 4°C.

The nuclear envelope fraction was collected as below. HepG2 cells grown in two 75 cm^2 flasks containing Git medium for 8 days were homogenized with a Potter-Elvehjem Teflon homogenizer in 2.0 ml of ice-cold 250 mM sucrose–25 mM KCl–5 mM $MgCl_2$–50 mM Tris-HCl isoosmolaric buffer (pH 7.5) (250 mM sucrose-TKM) as above. A549 cells grown in two 75 cm^2 flasks containing Git medium for 13 days were homogenized with a Polytron-homogenizer in 2.0 ml of ice-cold 250 mM sucrose–TKM for 1 min. Each homogenate was fractionated by centrifugation at 700 ×*g* for 10 min at 4°C. The pellet was suspended in 2.0 ml of ice-cold 250 mM sucrose-TKM, and then the suspension was fractionated by similar centrifugation. The resulting pellet was suspended in 0.5 ml of ice-cold ρ = 1.15 sucrose-TKM, and then the suspension was fractionated by sucrose density gradient centrifugation (ρ = 1.15, 1.20, and 1.30 sucrose-TKM) at 100,000 ×*g* for 2 h at 4°C. The band (nucleus fraction) at the ρ = 1.20–1.30 interface was collected and suspended in 2 ml of 250 mM sucrose-TKM. The suspension was fractionated by centrifugation at 100,000 ×*g* for 30 min at 4°C. The pellet was suspended in 1.5 ml of ice-cold 250 mM sucrose-TKM, and then 150 mg tripotassium citrate was dissolved in the suspension. In other cases, a 250 mM sucrose-TKM suspension of a pellet (nucleus fraction) derived from A549 cells was treated with ultrasonic waves for 20 s before the addition of tripotassium citrate. The suspension was fractionated by centrifugation at 100,000 ×*g* for 30 min at 4°C, and the pellet was suspended in 1.0 ml of ice-cold ρ = 1.20 sucrose-TKM–100 mg/ml tripotassium citrate. The suspension was fractionated by sucrose density gradient centrifugation (ρ = 1.16 and 1.12 sucrose-TKM–100 mg/ml tripotassium citrate) at 100,000 ×*g* for 2 h at 4°C. The band (nuclear envelope fraction) at ρ = 1.12 was collected and suspended in 2 ml of ice-cold 250 mM sucrose-TKM. The suspension was fractionated by centrifugation at 100,000 ×*g* for 30 min at 4°C. The pellet was re-suspended in 2 ml of ice-cold 250 mM sucrose-TKM, and then the suspension was re-fractionated by similar centrifugation. A nuclear envelope fraction of genistein-treated HepG2 or A549 cells was obtained with HepG2 or A549 cells grown in Git medium containing 75 μM genistein for 24 h before removal of the cells. DNA was measured with a DyNA Quant 200 (Pharmacia Biotech, USA) using Hoechst 33258.

ω-Hydroxylation Activity—For measurement of ω-hydroxylation activity in HepG2 or A549 cells, a homogenate of cells (1.0 ml each), a substrate [14.8 nmol LTB_4, 152 nmol 22:6(*n*-3), 164 nmol 20:4(*n*-6), 157 nmol 16:0, or 158 nmol 12:0] and NADPH (1 mg) were mixed with a vortex-mixer. To assess the pH dependence of the ω-hydroxylation activity, aliquots (0.1 ml each) of a homogenate, 14.8 nmol LTB_4, NADPH (1 mg), and 0.9 ml of 50 mM Tris-HCl buffer (pH 7.0–9.0) were mixed with a vortex-mixer. Each mixture was incubated at 37°C for 30 min under aerobic conditions. Each incubation mixture was acidified to *ca.* pH 4 with one-drop of 15% formic acid. Prostaglandin F_2 α-3,3,4,4-$^2H_4$ (278 pmol), for LTB_4 ω-hydroxylation activity or 15(*S*)hydroxyeicosatetraenoic acid-5,6,8,9,11,12,14,15-$^2H_8$ (304 pmol), for fatty acid ω-hydroxylation activity, was then added as an internal standard and the system was extracted two times with ethyl acetate. The ethyl acetate layer was dried under anhydrous sodium sulfate for 1 min and then filtered with filter paper. The filtrate was evaporated to dryness under reduced pressure. For measurement of fatty acid ω-hydroxylation activity, the residue was dissolved in 100 μl acetonitrile and then 20 μl aliquots were examined with an HPLC-thermospray-mass spectrometer. Reverse phase-HPLC separation was carried out on a Pegasil-ODS column (150 × 4.6 mm I.D.; Senshu Scientific, Tokyo), with a mobile phase of 0.1 M ammonium formate–0.1 M formic acid–acetonitrile (4:1:15) at the flow rate of 1.0 ml/min. For HPLC-thermospray mass spectrometry, the thermospray interface and mass spectrometer conditions were the same as previously reported (*11*, *13*, *14*). ω-Hydroxydodecanoic acid, ω-hydroxyeicosatetraenoic acid, ω-hydroxydocosahexaenoic acid and 15(*S*)-hydroxyeicosatetraenoic acid-5,6,8,9,11,12,14,15-$^2H_8$ (internal standard) appeared at retention times of 2.2, 4.1, 4.3, and 4.9 min, respectively. The analysis required about 6 min. The ω-1 or ω-hydroxyhexadecanoic acid as a palmitic acid ω-1 or ω-hydroxylation product appeared as ions MH^+-H_2O (*m*/*z* 255), MH^+ (*m*/*z* 273), MNH_4^+ (*m*/*z* 290), and MNa^+ (*m*/*z* 295) at a retention time of 4.5 or 4.8 min under the same HPLC-thermospray–mass spectrometry conditions.

ω-Carboxyeicosatetraenoic acid and ω-carboxydocosahexaenoic acid appeared as ions MH^+ (*m*/*z* 335, 359), MNH_4^+ (*m*/*z* 352, 376), and MNa^+ (*m*/*z* 357, 381) at retention times shorter than those for the corresponding ω-hydroxy-derivatives, respectively. 14,15-Epoxyeicosatrienoic acid, 11,12-epoxyeicosatrienoic acid, and 8,9-epoxyeicosatrienoic acid (*15*) appeared at retention times of 7.4, 8.3, and 8.7 min, respectively.

For measurement of LTB_4 ω-hydroxylation activity, the residue of an ethyl acetate extract was derivatized using acetic anhydride (80 μl) in pyridine (200 μl) under argon at 5°C overnight to obtain an acetic esters, which were then partially purified on a Sep-Pak C_{18} cartridge as previously described (*12*). HPLC-thermospray-mass spectrometry was performed in the same manner as decribed above. The ω-hydroxy-LTB_4 acetyl derivative, ω-carboxy-LTB_4 acetyl derivative, and prostaglandin F_2 α-3,3,4,4-$^2H_4$ acetyl derivative (internal standard) appeared at retention times of 4.6, 2.5, and 4.3 min, respectively.

For measurement of ω-hydroxylation activity in each subcellular fraction, the pellet obtained on differential centrifugation or sucrose density gradient centrifugation was suspended in 1.5 ml of ice-cold 50 mM Tris-HCl buffer (pH 8.0), and then ω-hydroxylation activity in each suspension was measured as described above. To examine the effects of CO gas, metyrapone and NADPH, a suspension (1.0 ml) of the nuclear envelope fraction of HepG2 or A549 cells was incubated under a CO gas phase with 1.1 mM NADPH, or 1 mM metyrapone with 1.1 mM NADPH or without NADPH using LTB_4 (14.8 nmol) as the substrate. To examine the effect of genistein in the enzyme assay medium, a suspension (1.0 ml) of the nuclear envelope fraction of A549 cells was incubated with 0–52.9 μM genistein with 1.1 mM NADPH, using 164 nmol of 20:4(*n*-6) as the substrate. In kinetic experiments, 20:4(*n*-6) or LTB_4 was varied from 2.9 to 32.8 μM.

For measurement of the amount of genistein contaminating the nuclear envelope fraction of 75 μM genistein-treated A549 cells, a suspension (1.0 ml) of the nuclear envelope fraction was acidified with one-drop of 15% formic acid and then mixed with 15(*S*)hydroxyeicosatetraenoic acid-5,6,8,9,11,12,14,15-$^2H_8$ (304 pmol) as an internal standard, and then the system was extracted two times with ethyl acetate. The ethyl acetate layer was dried, filtered and then evaporated, and the residue was subjected to HPLC-thermospray-mass spectrometry as described above. Genistein was characterized by the high intensity of the quasimolecular ion [MH^+ (*m*/*z* 271)] at the retention time of 2.0 min on selected-ion monitoring chromatography.

Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR) for CYP4F2 and CYP4A11—Total RNA was isolated from HepG2 cells at 7 days subculture in Git medium or A549 cells at 14 days subculture in Git medium by the acid guanidinium thiocyanate-phenol-chloroform method. The first strand cDNA was synthesized from 5 μg of RNA using Molony murine leukemia virus reverse transcriptase (Gibco BRL, Tokyo) and oligo(dT)-primers. The RT-PCR primers for CYP4F2 were EX1-D and EX3-U2, as described by Kikuta *et al.* (*16*). The RT-PCR primers for CYP4A11 were described by Bell *et al.* (*17*). The conditions for RT-PCR were 30 cycles for denaturing at 94°C for 1 min, primer annealing at 55°C for 1 min and primer extension at 72°C for 2 min. The RT-PCR fragments were analyzed by electrophoresis on 3.5% agarose gels. The expected amplified DNA fragment for CYP4F2 was 371 bp. The DNA fragment for CYP4A11 was 1,300 bp.

Marker Enzyme Activity in the Subcellular Fraction—Acid-phosphatase activity in the suspension of each pellet obtained on differential centrifugation was determined by the phenylphosphate method using an AcP K II Test Wako kit as specified by Wako Pure Chemical Industries. For measurement of glucose-6-phosphate (G6P) phosphatase or 5′-nucleotidase activity, 20 μl of each suspension, and 20 μl of 10 mM D-glucose-6-phosphate in 50 mM Tris-HCl buffer (pH 7.0) or 10 mM adenosine-5′-monophosphate in 50 mM Tris-HCl buffer (pH 8.0) were incubated for 1 h at 37°C. Inorganic phosphate was determined by the molybdate-blue method using a P-Test Wako Kit as specified by Wako Pure Chemical Industries.

RESULTS

Alteration of LTB_4 ω-Hydroxylation Activity in Cell Homogenates—LTB_4 ω-hydroxylation activity in HepG2 or A549 cell homogenates at day 8 in Eagle's MEM medium was maximal at pH 7.5–8.0 in 50 mM Tris-HCl buffer. The

effects of culture media on LTB_4 ω-hydroxylation activity are shown in Table I. Certain components in T-medium caused an increase in activity. Ten percent SGF had no effect, and glucose was shown to be very powerful on the screening of all agents in T-medium. The addition of glucose to the culture medium (Eagle's MEM medium) for 22 h caused a dose-dependent increase in LTB_4 ω-hydroxylation activity, as shown in Fig. 1. The conversion of ω-hydroxy-LTB_4 to ω-carboxy-LTB_4 in homogenates of HepG2 or A549 cells cultured in Git medium was negligibly low compared with the corresponding ω-hydroxylation activity.

The addition of genistein or sodium orthovanadate to the culture medium (Git medium) of HepG2 or A549 cells caused a dose-dependent decrease in LTB_4 ω-hydroxylation activity, as shown in Figs. 2 and 3. The number of living HepG2 or A549 cells was not influenced by the addition of genistein (Fig. 2). As shown in Table II, the inhibition (89–91% decrease) by 60 μM genistein of 20:4(*n*-6)/LTB_4 ω-hydroxylation activity was reversed to a 18–26% decrease by removal of genistein from the culture medium in 22 h. LTB_4 ω-hydroxylation activity in HepG2 or A549 cells was not influenced by 5 μM insulin, 100 nM glucagon, 10 μM *R*(–)epinephrine or 61 μM β-estradiol in the culture medium (Git medium).

Subcellular Localization of LTB_4 ω-Hydroxylation Activity—LTB_4 ω-hydroxylation activity, acid phosphatase activity, glucose-6-phosphatase activity, 5′-nucleotidase activity, and the DNA content in each subcellular fraction and each fraction obtained on sucrose density gradient centrifugation (ρ = 1.15–1.20) of HepG2 or A549 cells are shown in Tables III and IV. The high acid-phosphatase activities in the 7,000 ×g pellet fraction and ρ = 1.16–1.17 fraction of

TABLE I. **The effects of the culture media for HepG2 and A549 cells on LTB_4 ω-hydroxylation activity.** At 7 days (HepG2) or 13 days (A549) subculture, Eagle's MEM medium containing 10% FCS was replaced by fresh Eagle's MEM containing 10% FCS, or Git containing 10% SGF for 22 h. HepG2 or A549 cells were mechanically scraped off, washed and Polytron-homogenized. Each homogenate (1 ml) was incubated with LTB_4 (14.8 nmol) and NADPH (1 mg) at 37°C for 30 min under aerobic conditions. The data represent the means ± SD of triplicate measurements.

	LTB_4 ω-hydroxylation activity (pmol/min/mg protein)	
	Eagle's MEM containing 10% FCS	Git containing 10% SGF
HepG2 cell homogenate	6.1 ± 0.5	17.6 ± 2.1
A549 cell homogenate	21.3 ± 0.4	33.9 ± 2.3

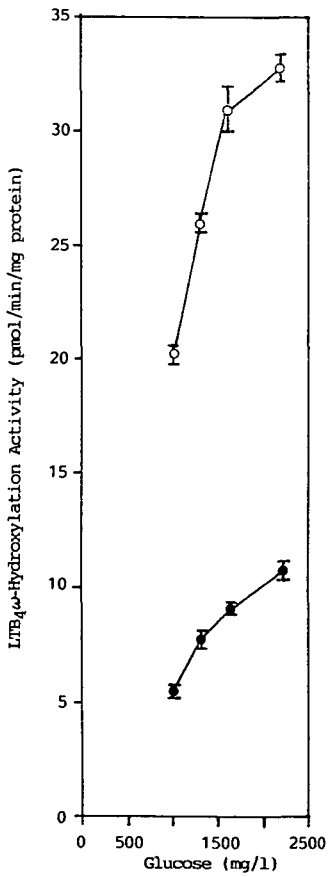

Fig. 1. **Effect of the glucose concentration in the culture medium (Eagle's MEM medium) on LTB_4 ω-hydroxylation activity of HepG2 or A549 cells.** At 22 h after glucose addition, the cells were scraped off and homogenized. The homogenate (1.0 ml), 14.8 nmol of LTB_4, and 1 mg of NADPH were mixed in a vortex-mixer. The mixture was incubated at 37°C for 30 min under aerobic conditions. ●, LTB_4 ω-hydroxylation activity in the HepG2 cell homogenate. ○, LTB_4 ω-hydroxylation activity in the A549 cell homogenate. The points are the means of three experiments. Vertical bars indicate SD.

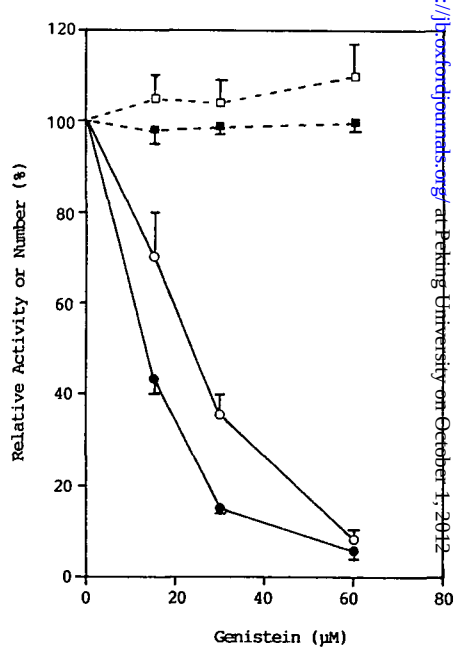

Fig. 2. **Effects of genistein in the culture medium (Git medium) on the number of living cells and LTB_4 ω-hydroxylation activity of HepG2 or A549 cells.** For determination of the number of living cells, a Cell Counting Kit-8 was used at 22 h after genistein addition. For measurement of ω-hydroxylation activity at 22 h after genistein addition, the cells were scraped off and homogenized. The homogenate (1.0 ml), 14.8 nmol of LTB_4, and 1 mg of NADPH were mixed in a vortex-mixer. The mixture was incubated at 37°C for 30 min under aerobic conditions. ●, LTB_4 ω-hydroxylation activity in the HepG2 cell homogenate. ○, LTB_4 ω-hydroxylation activity in the A549 cell homogenate. ■, the number of living HepG2 cells. □, the number of living A549 cells. The points are the means of three experiments. Vertical bars indicate SD.

the 7,000 ×*g* pellet fraction of HepG2/A549 cells likely depended on lysosome with mitochondria. The high G6P-phosphatase activities in the 7,000 ×*g* pellet fraction of HepG2/A549 cells, 100,000 ×*g* pellet fraction of A549 cells and ρ = 1.16 or 1.17 fraction of the 7,000 ×*g* pellet fraction of HepG2 or A549 cells likely depended on microsomes. The high 5'-nucleotidase activities in the 7,000 ×*g* pellet fraction of HepG2/A549 cells, 100,000 ×*g* pellet fraction of A549 cells and ρ = 1.15–1.17 fraction of the 7,000 ×*g* pellet fraction of HepG2/A549 cells likely depended on cell membranes. The DNA content was high in the nuclear envelope fraction and this content likely depended on DNA from nuclei. LTB_4 ω-hydroxylation activity per fraction of the 100 ×*g* pellet fractions of HepG2/A549 cells was dependent on the increase of ρ on sucrose density gradient centrifugation, and thus much of the activity was concentrated in the high density (ρ = 1.20) fraction based on nuclei. The specific LTB_4 ω-hydroxylation activity was high in the nuclear envelope fraction, and a large portion of the activity in HepG2 or A549 cells was concentrated in the nuclear envelope fraction. Although treatment of the nuclear fraction from A549 cells with ultrasonic waves for breakage of DNA fibers and nuclear envelope caused a decrease in the DNA content, the specific LTB_4 ω-hydroxylation activity of the nuclear envelope fraction showed a 35% reduction compared with no treatment (Tables IV and V). Homogenization with a Polytron homogenizer of HepG2 cells lowered the ω-hydroxylation activity in the nuclear envelope fraction and increased the activity in the 7,000 ×*g* pellet fraction. The homogenization perhaps causes partial disruption of nuclei in HepG2 cells.

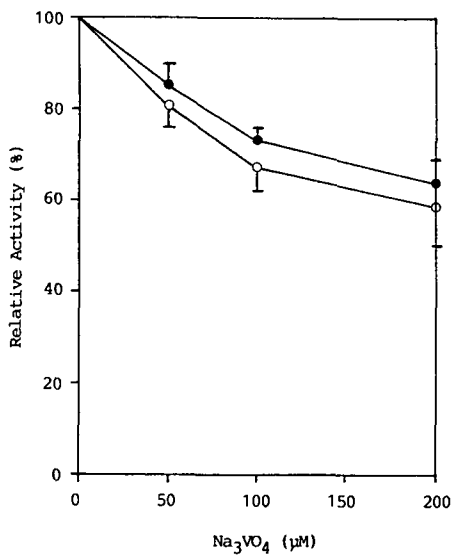

Fig. 3. **Effect of sodium orthovanadate in the culture medium (Git medium) on LTB_4 ω-hydroxylation activity of HepG2 or A549 cells.** At 22 h after sodium orthovanadate addition, the cells were scraped off and homogenized. The homogenate (1.0 ml), 14.8 nmol of LTB_4 and 1 mg of NADPH were mixed in a vortex-mixer. The mixture was incubated at 37°C for 30 min under aerobic conditions. ●, LTB_4 ω-hydroxylation activity in the HepG2 cell homogenate. ○, LTB_4 ω-hydroxylation activity in the A549 cell homogenate. The points are the means of three experiments. Vertical bars indicate SD.

ω-Hydroxylation Activity in the Nuclear Envelope Fraction—The substrate specificity and DNA content in the nuclear envelope fraction of HepG2 or A549 cells with/without genistein treatment are shown in Table V. High polyunsaturated fatty acid ω-hydroxylation activity with LTB_4 ω-hydroxylation activity was observed in the nuclear envelope fractions of HepG2 and A549 cells. Treatment with genistein for 24 h caused a marked decrease in ω-hydroxylation activity. The apparent K_m values for 20:4(*n*-6) and LTB_4 in the nuclear envelope fraction of HepG2 or A549 cells determined by means of Lineweaver-Burk plots, as shown in Fig. 4, were 25 and 50 μM, or 22 and 66 μM, respectively. The V_{max} values for 20:4(*n*-6) and LTB_4 in the nuclear envelope fraction of HepG2 or A549 cells were 222 and 104 pmol/min/mg protein, or 175 and 370 pmol/min/mg protein, respectively. When the nuclear envelope fraction of HepG2 or A549 cells was incubated under a CO gas phase with NADPH, or 1 mM metyrapone with NADPH or without NADPH with LTB_4 as the substrate, the LTB_4 ω-hydroxylation activity was 100% inhibited on incubation without NADPH and under the CO gas phase, and 69% (in HepG2 cells) or 47% (in A549 cells) inhibited on incubation with 1 mM metyrapone. When the nuclear envelope fraction of A549 cells was incubated with 0–59.2 μM genistein, with 164 μM 20:4(*n*-6) as the substrate, the 20:4(*n*-6) ω-hydroxylation activity was not influenced by 29.6 nM–2.96 μM genistein, although the activity was 86% inhibited by 59.2 μM genistein. The conversion of ω-hydroxy-LTB_4 to ω-carboxy-LTB_4 in the nuclear envelope fraction of HepG2 or A549 cells was negligibly low compared with the corresponding ω-hydroxylation activity. No 20:4(*n*-6)epoxygenase activity was detected in the nuclear envelope fraction of HepG2 or A549 cells. The genistein content in the nuclear envelope fraction of 75 μM genistein–treated A549 cells was 122 pmol/mg protein.

RT-PCR for CYP4F2 in HepG2 and A549 Cells—CYP4F2 mRNAs were detected in HepG2 and A549 cell samples as shown in Fig. 5. Although only trace levels of expression of CYP4A11 mRNA in HepG2 cell samples were detected compared with the expression of house-keeping glyceraldehyde-3-phosphate dehydrogenase mRNA, the expression of

TABLE II. **Restoration from genistein-inhibition of 20:4(*n*-6)/LTB_4 ω-hydroxylation activity in HepG2 cells.** HepG2 cells were grown in Git medium for 5 days after subculture, and 60 μM genistein was added for 22 h. For restoration, the culture medium was then replaced with only Git medium for 22 h. The cells were mechanically scraped off and Polytron-homogenized. The homogenate (1.0 ml) was incubated with 164 nmol 20:4(*n*-6) or 14.8 nmol LTB_4 and NADPH (1 mg) at 37°C for 30 min under aerobic conditions. The data represent the means ± SD of triplicate measurements.

	ω-Hydroxylation activity (pmol/min/mg protein)			
	20:4(*n*-6)	Relative activity (%)	LTB_4	Relative activity (%)
Control (6 days after subculture)	72.2 ± 2.3	100.0	20.7 ± 1.1	100.0
Genistein treatment (60 μM) for 22 h at 5 days	6.4 ± 0.7	8.8	2.1 ± 0.5	10.1
Control (7 days after subculture)	75.4 ± 1.8	100.0	18.0 ± 0.7	100.0
Restoration from genistein treatment for 22 h at 6 days	61.7 ± 8.9	81.8	13.3 ± 0.7	73.8

TABLE III. **Subcellular localization of LTB_4 ω-hydroxylation activity in HepG2 cells.** Each fraction obtained on sucrose density gradient centrifugation was suspended in 1.5 ml of 50 mM Tris-HCl buffer (pH 8.0). For measurement of LTB_4 ω-hydroxylation activity, the suspension (1.0 ml each) of each fraction was incubated with LTB_4 (14.8 nmol) and NADPH (1 mg) at 37°C for 30 min under aerobic condition. Acid-phosphatase activity in the suspension (50 μl each) of each fraction was determined by the phenylphosphate method using an AcP KII-Test Wako kit. For measurement of G6P-phosphatase or 5′-nucleotidase activity, 20 μl of each suspension and 20 μl of 10 mM D-glucose-6-phosphate in 50 mM Tris-HCl buffer (pH 7.0) or 10 mM adenosine-5′-monophosphate in 50 mM Tris-HCl buffer (pH 8.0) were incubated for 1 h at 37°C. Inorganic phosphate was determined by the molybdate-blue method using a P-Test Wako Kit. DNA was measured with a DyNA Quant 200 using Hoechst 33258.

Fraction	LTB_4 ω-hydroxylation		Acid-phosphatase	G6P-phosphatase	5′-Nucleotidase	DNA content
	pmol/min/mg protein	pmol/min/fraction	nmol/min/mg protein			μg/mg protein
Homogenate	13.1 ± 0.3[a]	213.4 ± 20.5[a]	18.3 ± 1.9[a]	6.2 ± 0.3[a]	15.8 ± 2.3[a]	60.1 ± 3.1[a]
Nucleus	24.6 ± 1.7[a]	169.1 ± 5.8[a]	15.7 ± 1.2[a]	5.6 ± 0.1[a]	21.9 ± 0.7[a]	83.1 ± 15.5[a]
Nuclear envelope	32.4 ± 2.5[a]	89.0 ± 4.3[a]	11.9 ± 1.5[a]	5.9 ± 0.5[a]	35.6 ± 4.1[a]	136.0 ± 15.1[a]
7,000 ×*g* pellet	19.3 ± 0.6[a]	46.2 ± 5.6[a]	16.8 ± 2.4[a]	7.3 ± 1.2[a]	47.3 ± 5.4[a]	43.3 ± 7.0[a]
100,000 ×*g* pellet	23.6 ± 0.2[a]	18.7 ± 0.4[a]	12.7 ± 2.2[a]	3.8 ± 0.3[a]	30.9 ± 4.0[a]	28.4 ± 13.9[a]
ρ < 1.15 from 100 ×*g* pellet	3.0[b]	0.8[b]	13.1[b]	0[b]	96.8[b]	16.5[b]
ρ = 1.16	2.2[b]	0.3[b]	6.0[b]	0[b]	36.3[b]	10.2[b]
ρ = 1.17	4.2[b]	0.9[b]	4.3[b]	2.4[b]	28.1[b]	7.4[b]
ρ = 1.18	10.7[b]	7.5[b]	1.8[b]	0[b]	9.1[b]	29.9[b]
ρ = 1.19	13.9[b]	55.9[b]	9.6[b]	6.2[b]	29.6[b]	74.1[b]
ρ = 1.20	21.5[b]	120.9[b]	13.5[b]	9.3[b]	37.6[b]	106.8[b]
ρ < 1.15 from 7,000 ×*g* pellet	18.5[b]	4.5[b]	15.5[b]	13.5[b]	121.1[b]	0[b]
ρ = 1.16	0[b]	0[b]	20.0[b]	12.4[b]	157.2[b]	35.7[b]
ρ = 1.17	0[b]	0[b]	7.4[b]	4.9[b]	67.7[b]	61.6[b]
ρ = 1.18	0[b]	0[b]	3.1[b]	1.7[b]	30.8[b]	12.8[b]
ρ = 1.19	10.8[b]	6.7[b]	7.1[b]	3.5[b]	70.0[b]	23.9[b]
ρ = 1.20	15.3[b]	24.1[b]	9.5[b]	8.1[b]	56.6[b]	52.0[b]

[a]Mean ± SD of triplicate measurements. [b]Mean of duplicate determinations.

TABLE IV. **Subcellular localization of LTB_4 ω-hydroxylation activity in A549 cells.** Each fraction obtained on sucrose density gradient centrifugation was suspended in 1.5 ml of 50 mM Tris-HCl buffer (pH 8.0). For measurement of LTB_4 ω-hydroxylation activity, the suspension (1.0 ml each) of each fraction was incubated with LTB_4 (14.8 nmol) and NADPH (1 mg) at 37°C for 30 min under aerobic conditions. Acid-phosphatase activity in the suspension (50 μl each) of each fraction was determined by the phenylphosphate method using an AcP KII-Test Wako kit. For measurement of G6P-phosphatase or 5′-nucleotidase activity, 20 μl of each suspension and 20 μl of 10 mM D-glucose-6-phosphate in 50 mM Tris-HCl buffer (pH 7.0) or 10 mM adenosine-5′-monophosphate in 50 mM Tris-HCl buffer (pH 8.0) were incubated for 1 h at 37°C. Inorganic phosphate was determined by the molybdate-blue method using a P-Test Wako Kit. DNA was measured with a DyNA Quant 200 using Hoechst 33258.

Fraction	LTB_4 ω-hydroxylation		Acid-phosphatase	G6P-phosphatase	5′-Nucleotidase	DNA content
	pmol/min/mg protein	pmol/min/fraction	nmol/min/mg protein			μg/mg protein
Homogenate	26.9 ± 0.2[a]	566.1 ± 22.5[a]	25.1 ± 1.7[a]	8.1 ± 0.5[a]	71.2 ± 1.3[a]	30.0 ± 4.0[a]
Nucleus	41.4 ± 0.2[a]	187.6 ± 25.0[a]	20.3 ± 0.9[a]	7.0 ± 1.2[a]	76.0 ± 1.4[a]	42.6 ± 5.9[a]
Nuclear envelope	109.0 ± 2.4[a]	151.3 ± 18.1[a]	15.5 ± 2.0[a]	4.7 ± 0.1[a]	67.0 ± 7.6[a]	73.3 ± 11.2[a]
7,000 ×*g* pellet	7.9 ± 1.8[a]	5.7 ± 2.8[a]	24.2 ± 2.4[a]	7.9 ± 1.4[a]	300.8 ± 43.4[a]	18.2 ± 7.2[a]
100,000 ×*g* pellet	4.8 ± 0.3[a]	1.9 ± 0.5[a]	15.9 ± 0.2[a]	11.3 ± 2.4[a]	432.5 ± 98.5[a]	10.1 ± 3.8[a]
ρ < 1.15 from 100 ×*g* pellet	0[b]	0[b]	5.7[b]	0[b]	156.4[b]	11.2[b]
ρ = 1.16	0[b]	0[b]	11.7[b]	0[b]	266.5[b]	22.9[b]
ρ = 1.17	0[b]	0[b]	0[b]	0[b]	292.0[b]	31.2[b]
ρ = 1.18	40.0[b]	3.0[b]	13.6[b]	0[b]	32.3[b]	68.7[b]
ρ = 1.19	32.4[b]	19.6[b]	14.8[b]	4.1[b]	46.8[b]	41.9[b]
ρ = 1.20	40.5[b]	200.6[b]	20.6[b]	7.4[b]	72.5[b]	51.6[b]
ρ < 1.15 from 7,000 ×*g* pellet	24.2[b]	2.8[b]	11.7[b]	0[b]	144.2[b]	91.4[b]
ρ = 1.16	0[b]	0[b]	19.5[b]	10.7[b]	274.4[b]	89.8[b]
ρ = 1.17	0[b]	0[b]	26.0[b]	129.1[b]	398.1[b]	203.3[b]
ρ = 1.18	0[b]	0[b]	17.0[b]	35.2[b]	158.7[b]	272.2[b]
ρ = 1.19	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]
ρ = 1.20	25.2[b]	6.2[b]	12.3[b]	12.8[b]	117.7[b]	29.8[b]

[a]Mean ± SD of triplicate measurements. [b]Mean of duplicate determinations.

CYP4A11 mRNA in A549 cell samples was not detected (data not shown).

DISCUSSION

A large portion of the LTB_4 ω-hydroxylation activity in HepG2 or A549 cells was concentrated in the nuclear envelope fraction, as shown in Tables III and IV, and this activity was accompanied by polyunsaturated fatty acid ω-hydroxylation activity (Table V). NADPH-dependent ω-hydroxylation of LTB_4 in the nuclear envelope fraction of HepG2 or A549 cells was strongly inhibited by metyrapone and CO. Thus, ω-hydroxylation is quite likely associated with CYP. On analysis by RT-PCR of expression of the mRNA of CYP4F2 or CYP4A11 in HepG2 and A549 cells, CYP4F2 mRNAs were detected in HepG2 and A549 cells (Fig. 5), although the presence of CYP4A11 mRNA in HepG2 and A549 cells was negligible. Furthermore, since the 12:0 or 16:0 ω-hydroxylation activity of the enzyme from HepG2 or A549 cells was low compared to that toward

TABLE V. **Substrate specificity and DNA content of the nuclear envelope fraction of HepG2 or A549 cells with/without genistein treatment.** The suspension (1 ml) of the nuclear envelope fraction was incubated with a precursor fatty acid [152 nmol 22:6(*n*-3), 164 nmol 20:4(*n*-6), 157 nmol 16:0, 158 nmol 12:0 or 14.8 nmol LTB_4] and NADPH (1 mg) at 37°C for 30 min under aerobic conditions. The data represent the means ± SD of triplicate measurements.

Cell	Fraction	ω-Hydroxylation activity (pmol/min/mg protein)					DNA content
		22:6(*n*-3)	20:4(*n*-6)	16:0	12:0	LTB_4	μg DNA/mg protein
HepG2	Nuclear envelope (without genistein treatment)	278 ± 39	209 ± 21	130 ± 13	67 ± 12	37 ± 5	148 ± 23
	Nuclear envelope (with genistein treatment)	6 ± 0.5	4 ± 0.3	2 ± 0.2	0	1 ± 0.1	144 ± 15
A549	Nuclear envelope (without genistein treatment)[a]	178 ± 19	97 ± 9	68 ± 9	31 ± 3	71 ± 13	27 ± 7
	Nuclear envelope (with genistein treatment)[a]	11 ± 1	7 ± 1	8 ± 2	0	11 ± 2	33 ± 8

[a]Different from in the case of HepG2 cells, the nucleus fraction was treated with ultrasonic waves for 20 s before the addition of tripotassium citrate.

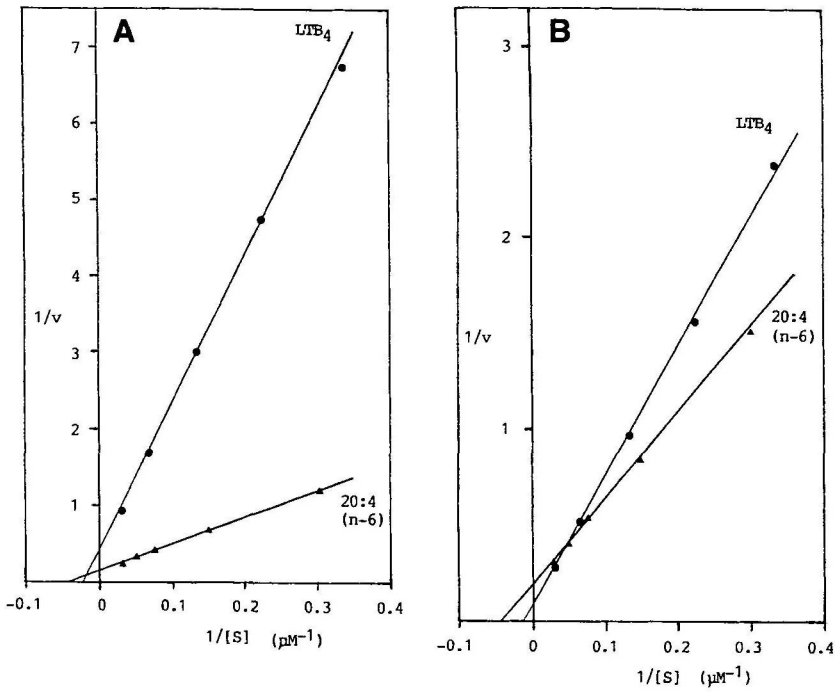

Fig. 4. **Lineweaver-Burk plots of Michaelis-Menten parameters for 20:4(*n*-6) or LTB_4 ω-hydroxylation in the nuclear envelope fraction of HepG2 or A549 cells.** (A) Nuclear envelope fraction of HepG2 cells; (B) nuclear envelope fraction of A549 cells. The suspension [0.1 ml (0.285 mg protein)] of the nuclear envelope fraction of HepG2 cells or that [0.1 ml (0.207 mg protein)] of A549 cells, a precursor fatty acid [3.24–32.4 nmol of 20:4(*n*-6) or 2.96–29.6 nmol of LTB_4], 0.9 ml of 50 mM Tris-HCl buffer (pH 8.0), and 1 mg of NADPH were mixed in a vortex-mixer. The mixture was incubated at 37°C for 30 min under aerobic conditions. Data shown for 20:4(*n*-6) (▲) and LTB_4 (●). The apparent K_m values for 20:4(*n*-6) and LTB_4 in the nuclear envelope fraction of HepG2 or A549 cells were 25 and 50 μM, or 22 and 66 μM, respectively. The V_{max} values for 20:4(*n*-6) and LTB_4 in the nuclear envelope fraction of HepG2 or A549 cells were 222 and 104 pmol/min/mg protein, or 175 and 370 pmol/min/mg protein, respectively.

22:6(*n*-3) and 20:4(*n*-6) (Table V), its substrate specificity clearly differs from CYP4A11.

Reports by Kikuta *et al.* showed that no activity was detected with 12:0 or 20:4(*n*-6) in microsomes from yeast transfected with CYP4F2 cDNA (*6*, *7*), and no 12:0 ω-hydroxylation activity was detected in a HepG2 cell homogenate although expression of the CYP4F2 gene has been shown in HepG2 cells (*16*). In contrast, the CYP4F2 purified from human liver microsomes by Powell *et al.* was found to catalyze the ω-hydroxylation of not only LTB_4 but also 20:4(*n*-6) (*8*, *9*). Although the reason for this discrepancy is presently unknown, the CYP4F2 from human liver microsomes shows ω-hydroxylation activity toward 20:4(*n*-6) and LTB_4 with apparent K_m values of 23 and 74 μM (*8*, *9*), and thus the ω-hydroxylation activity [apparent K_m values (22–25 and 50–66 μM) for 20:4(*n*-6) and LTB_4] in the nuclear envelope fraction of HepG2 or A549 cells is likely due to CYP4F2. Furthermore, as the K_m value of CYP4F2 for LTB_4 is 94-fold higher than that of CYP4F3 (0.64 μM) from human neutrophils (*7*), the activity in the nuclear envelope fraction would not be due to CYP4F3. On the other hand, since human lung CYP4B1 is unable to metabolize 12:0 or 20:4(*n*-6) (*18*) and there is no detectable amount of CYP4B1 mRNA in human liver (*19*), the ω-hydroxylation activity of HepG2 or A549 cells would not be due to CYP4B1.

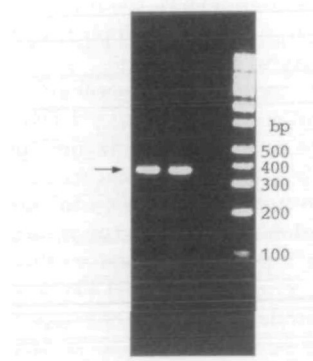

Fig. 5. **Expression of CYP4F2 in HepG2 and A549 cells.** The PCR product is indicated by an arrow on the left and the sizes of amplified DNA fragments are given in base pairs on the right (CYP4F2 = 371). Lane 1, the sample from HepG2 cells; lane 2, the sample from A549 cells; and lane 3, 100 bp DNA ladder as molecular markers.

The change from Eagle's MEM medium to Git medium

caused enhancement of the LTB_4 ω-hydroxylation activity in HepG2 or A549 cells (Table I). Although the main factor for the activation was glucose concentration in the medium (Fig. 1), various factors, such as putrescine, vitamins and amino acids, contributed to the activation (data not shown). Although the dose-dependent increase or decrease (Fig. 1 or 2) of the activity on the addition of glucose or genistein (tyrosine-kinase inhibitor) indicates modulation of the activity was associated with tyrosine-kinase–dependent systems (*20*), genistein is a potent inhibitor of glucose transport (*21*) and inhibits insulin-stimulated glucose oxidation without affecting insulin receptor autophosphorylation (*22*, *23*). Judging from the dose-dependent decrease (Fig. 3) in the activity caused by sodium orthovanadate, a tyrosine-phosphatase inhibitor (*24*), the involvement of tyrosine-kinase–dependent systems in the activity appears extremely doubtful. The LTB_4 ω-hydroxylation activity in HepG2 or A549 cells was not affected by insulin (5 μM), glucagon (100 nM), *R*(−)epinephrine (10 μM), or β-estradiol (61 μM) in 22 h. The reversible inhibition (Table II) by genistein of the activity may thus be explained by direct inhibition of a glucose transporter. In rat, increases in plasma glucose and hepatic CYP4A1 family proteins in streptozotocin-induced diabetes (*25*) may possibly result from an increase in direct glucose transport by the glucose transporter in rat hepatocells. On the other hand, although genistein is known as a β-estradiol–like cancer-prevention agent causing growth-inhibition, the number of living HepG2 or A549 cells was not influenced by the addition of genistein (Fig. 2), and thus the decrease in LTB_4 ω-hydroxylation activity caused by genistein may not be due to a growth-inhibition function of β-estradiol. On the other hand, CYP-mediated reactions were inhibited by flavonoids such as quercetin in an enzyme assay medium (*26*), and 20:4(*n*-6) ω-hydroxylation activity was, in fact, 86% inhibited by 59.2 μM genistein. However, since the activity was not influenced by 29.6 nM–2.96 μM genistein and the genistein content in the enzyme assay medium for the nuclear envelope fraction of 75 μM genistein–treated A549 cells was always less than 30 nM, the influence of genistein contamination of the nuclear envelope fraction on the ω-hydroxylation activity was negligible.

LTB_4 or 20:4(*n*-6) epoxides may possibly serve as intracellular second messengers (*27–29*). LTB_4 biosynthesis takes place through a complex of enzymes present in the nuclear envelope (*29*), and LTB_4 acts as an intracellular/intranuclear second messenger related to signal transduction or gene regulation *via* not only the receptor in the cell membrane, but also the nuclear receptor (*30*). The fact that LTB_4 ω-hydroxylation activity, as an LTB_4 inactivation system, actually surrounds the nuclear envelope is interesting.

In summary, we have shown that in HepG2 or A549 cells, a large portion of LTB_4 ω-hydroxylation activity likely based on CYP4F2 is concentrated in the nuclear envelope fraction and that the activity is accompanied by high polyunsaturated fatty acid ω-hydroxylation activity. The addition of glucose or genistein to the culture medium of the cells caused a dose-dependent increase or decrease in the activity in the homogenate. We are interested in elucidating the physiological function of the activity in the nuclear envelope fraction.

REFERENCES

1. McGiff, J.C. (1991) Cytochrome P450-dependent metabolism of arachidonic acid. *Annu. Rev. Pharmacol. Toxicol.* **31**, 339–369
2. Rahman, M., Wright, J.J., and Douglas, J. (1997) The role of the cytochrome P450-dependent metabolites of arachidonic acid in blood pressure regulation and renal function. *Am. J. Hypertens.* **10**, 356–365
3. Palmer, C.N.A., Richardson, T.H., Griffin, K.J., Hsu, M.H., Muerhoff, A.S., Clark, J.E., and Johnson, E.F. (1993) Characterization of a cDNA encoding a human kidney, cytochrome P4504A fatty acid ω-hydroxylase and the cognate enzyme expressed in *Escherichia coli*. *Biochim. Biophys. Acta* **1172**, 161–166
4. Kawashima, H., Kusunose, E., Kikuta, Y., Kinoshita, H., Tanaka, S., Yamamoto, S., Kishimoto, T., and Kusunose, M. (1994) Purification and cDNA cloning of human liver CYP4A fatty acid ω-hydroxylase. *J. Biochem.* **116**, 74–80
5. Hoch, U., Zhang, Z., Kroetz, D.L., and Montellano, P.R.O. (2000) Structural determination of the substrate specificities and regioselectivities of the rat and human fatty acid ω-hydroxylases. *Arch. Biochem. Biophys.* **373**, 63–71
6. Kikuta, Y., Kusunose, E., Kondo, T., Yamamoto, S., Kinoshita, H., and Kusunose, M. (1994) Cloning and expression of a novel form of leukotriene B_4 ω-hydroxylase from human liver. *FEBS Lett.* **348**, 70–74
7. Kikuta, Y., Kusunose, E., and Kusunose, M. (2000) Characterization of human liver leukotriene B_4 ω-hydroxylase P450 (CYP4F2). *J. Biochem.* **127**, 1047–1052
8. Powell, P.K., Wolf, I., Jin, R., and Lasker, J.M. (1998) Metabolism of arachidonic acid to 20-hydroxy-5,8,11,14-eicosatetraenoic acid by P450 enzymes in human liver: involvement of CYP4F2 and CYP4A11. *J. Pharmacol. Exp. Ther.* **285**, 1327–1336
9. Jin, R., Koop, D.R., Raucy, J.L., and Lasker, J.M. (1998) Role of human CYP4F2 in hepatic catabolism of the proinflammatory agent leukotriene B4. *Arch. Biochem. Biophys.* **359**, 89–98
10. Shimizu, S., Yamane, M., Abe, A., Nakajima, M., Sugiura, H., Miyaoka, M., and Saitoh, T. (1995) ω-Hydroxylation of docosahexaenoic acid or arachidonic acid in human colonic well-differentiated adenocarcinoma homogenate. *Biochim. Biophys. Acta* **1256**, 293–296
11. Yamane, M., Shimizu, S., Abe, A., and Yamane, S. (1998) Docosahexaenoic/arachidonic acid ω-hydroxylation system and differentiation in the human colonic adenocarcinoma cell line, Caco-2. *Cancer Lett.* **122**, 51–59
12. Yamane, M., Shimizu, S., Abe, A., Sugiura, H., Miyaoka, M., and Saitoh, T. (1995) High-performance liquid chromatography-thermospray mass spectrometry of ω-carboxyleukotriene B_4 and ω-hydroxyleukotriene B_4 from an incubation mixture of human colonic well-differentiated adenocarcinoma homogenate. *J. Chromatogr. B* **666**, 197–202
13. Yamane, M., Abe, A., Shimizu, S., and Nakajima, M. (1996) Subcellular localization of docosahexaenoic acid and arachidonic acid ω-hydroxylation activity in the brain, liver and colonic adenocarcinoma. *J. Chromatogr. A* **730**, 91–98
14. Yamane, M., Abe, A., and Nakajima, M. (1994) High-performance liquid chromatography-thermospray mass spectrometry of ω-hydroxy polyunsaturated fatty acids from rat brain homogenate. *J. Chromatogr. B* **662**, 91–96
15. Yamane, M., Abe, A., and Yamane, S. (1994) High-performance liquid chromatography-thermospray mass spectrometry of epoxy polyunsaturated fatty acids and epoxyhydroxy polyunsaturated fatty acids from an incubation mixture of rat tissue homogenate. *J. Chromatogr. B* **652**, 123–136
16. Kikuta, Y., Miyauchi, Y., Kusunose, E., and Kusunose, M. (1999) Expression and molecular cloning of human liver leukotriene B_4 ω-hydroxylase (CYP4F2) gene. *DNA Cell Biol.* **18**, 723–730
17. Bell, D.R., Plant, N.J., Rider, C.G., Na, L., Brown, S., Ateitalla, I., Acharya, S.K., Davies, M.H., Elias, E., Jenkins, N.A., Gilbert, D.J., Copeland, N.G., and Elcombe, C.R. (1993) Species-specific

induction of cytochrome P-450 4A RNAs: PCR cloning of partial guinea-pig, human and mouse CYP4A cDNAs. *Biochem. J.* **294**, 173–180
18. Imaoka, S., Asai, S., Sugimoto, T., Hiroi, T., and Funae, Y. (1999) Comparison of human CYP4B1 with that of other species in *11th Int. Confer. on Cytochrome P450*, p. 148, abstract
19. Nhamburo, P.T., Gonzalez, F.J., McBride, O.W., Gelboin, H.V., and Kimura, S. (1989) Identification of a new P450 expressed in human lung: complete cDNA sequence, cDNA-directed expression, and chromosome mapping. *Biochemistry* **28**, 8060–8066
20. Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M., and Fukami, Y. (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J. Biol. Chem.* **262**, 5592–5595
21. LeFevre, P.G. and Marshall, J.K. (1959) The attachment of phloretin and analogues to human erythrocytes in connection with inhibition of sugar transport. *J. Biol. Chem.* **234**, 3022–3026
22. Abler, A., Smith, J.A., Randazzo, P.A., Rothenberg, P., and Jarett, L. (1992) Genistein differentially inhibits postreceptor effects of insulin in rat adipocytes without inhibiting the insulin receptor kinase. *J. Biol. Chem.* **267**, 3946–3951
23. Young, S.W., Poole, R.C., Hudson, A.T., Halestrap, A.P., Denton, R.M., and Tavare, J.M. (1993) Effects of tyrosine kinase inhibitors on protein kinase-independent systems. *FEBS Lett.* **316**, 278–282
24. Hamaguchi, T., Sudo, T., and Osada, H. (1995) RK-682, a potent inhibitor of tyrosine phosphatase, arrested the mammalian cell cycle progression at G_1 phase. *FEBS Lett.* **372**, 54–58
25. Barnett, C.R., Gibson, G.G., Wolf, C.R., Flatt, P.R., and Ioannides, C. (1990) Induction of cytochrome P450 III and P450 IV family proteins in streptozotocin-induced diabetes. *Biochem. J.* **268**, 765–769
26. Sousa, R.L. and Marletta, M.A. (1985) Inhibition of cytochrome P-450 activity in rat liver microsomes by the naturally occurring flavonoid, Quercetin. *Arch. Biochem. Biophys.* **240**, 345–357
27. Makita, K., Falck, J.R., and Capdevila, J. (1996) Cytochrome P450, the arachidonic acid cascade, and hypertension: new vistas for an old enzyme system. *FASEB J.* **10**, 1456–1463
28. Chen, J.K., Wang, D.W., Falck, J.R., Capdevila, J., and Harris, R.C. (1999) Transfection of an active cytochrome P450 arachidonic acid epoxygenase indicates that 14,15-epoxyeicosatrienoic acid functions as an intracellular second messenger in response to epidermal growth factor. *J. Biol Chem.* **274**, 4764–4769
29. Serhan, C.N., Haeggstrom, J.Z., and Leslie, C.C. (1996) Lipid mediator networks in cell signaling: update and impact of cytokines. *FASEB J.* **10**, 1147–1158
30. Devchand, P.R., Keller, H., Peters, J.M., Vazquez, M., Gonzalez, F.J., and Wahli, W. (1996) The PPAR α-leukotriene B_4 pathway to inflammation control. *Nature* **384**, 39–43